

WHAT IS CLAIMED IS:

- 1 1. An isolated infectious recombinant respiratory syncytial virus (RSV)
2 comprising a RSV genome or antigenome, a major nucleocapsid (N) protein, a
3 nucleocapsid phosphoprotein (P), a large polymerase protein (L), and a RNA polymerase
4 elongation factor, wherein a modification is introduced in the genome or antigenome
5 comprising a partial or complete deletion of M2 ORF2 or one or more nucleotide
6 change(s) that reduce or ablate expression of M2 ORF2.
- 1 2. The recombinant RSV of claim 1, wherein expression of M2 ORF2 is
2 ablated by introduction of one or more stop codons.
- 1 3. The recombinant RSV of claim 2 which is rA2-K5.
- 1 4. The recombinant RSV of claim 1, wherein expression of M2 ORF2 is
2 ablated by introduction of a frame shift mutation.
- 1 5. The recombinant RSV of claim 4 which is rA2-NdeI.
- 1 6. The recombinant RSV of claim 1, wherein M2 ORF2 is deleted in whole
2 or in part.
- 1 7. The recombinant RSV of claim 1, wherein the modification in the genome
2 or antigenome specifies one or more desired phenotypic changes in the recombinant RSV
3 selected from (i) a change in mRNA synthesis, (ii) a change in the level of viral protein
4 expression; (iii) a change in genomic or antigenomic RNA replication, (iv) a change in
5 viral growth characteristics, (v), a change in viral plaque size, and/or (vi) a change in
6 cytopathogenicity.
- 1 8. The recombinant RSV of claim 7, wherein the phenotypic change
2 comprises attenuation of viral growth compared to growth of a corresponding wild-type
3 or mutant parental RSV strain.
- 1 9. The recombinant RSV of claim 1, wherein the RSV genome comprises one
2 or more shifted RSV gene(s) or genome segment(s) that is/are positionally shifted within
3 genome or antigenome to a more promoter-proximal or promoter-distal position relative

4 to a position of said RSV gene(s) or genome segment(s) within a wild type RSV genome
5 or antigenome.

1 10. The recombinant RSV of claim 9, wherein said one or more shifted
2 gene(s) or genome segment(s) is/are shifted to a more promoter-proximal or promoter-
3 distal position by deletion or insertion of one or more displacement polynucleotide(s)
4 within said partial or complete genome or antigenome.

1 11. The recombinant RSV of claim 7, wherein the phenotypic change
2 comprises delayed kinetics of viral mRNA synthesis compared to kinetics of mRNA
3 synthesis of a corresponding wild-type or mutant parental RSV strain.

1 12. The recombinant RSV of claim 7, wherein the phenotypic change
2 comprises a change in cumulative mRNA synthesis compared to cumulative mRNA
3 synthesis of a corresponding wild-type or mutant parental RSV strain.

1 13. The recombinant RSV of claim 12, wherein the increase in cumulative
2 viral mRNA synthesis is approximately 1.3 to 2-fold or greater at 24 hours post-infection
3 compared to cumulative mRNA synthesis of the corresponding wild-type or mutant
4 parental RSV strain.

1 14. The recombinant RSV of claim 7, wherein the phenotypic change
2 comprises increased viral protein accumulation in infected cells compared to viral protein
3 accumulation in cells infected with a corresponding wild-type or mutant parental RSV
4 strain.

1 15. The recombinant RSV of claim 7, wherein accumulation of one or more
2 viral proteins is increased approximately 2- to 3-fold or greater compared to viral protein
3 accumulation in cells infected with the corresponding wild-type or mutant parental RSV
4 strain.

1 16. The recombinant RSV of claim 7, wherein the phenotypic change
2 comprises increased expression of one or more viral antigens compared to expression of
3 said one or more viral antigens by the corresponding wild-type or mutant parental RSV
4 strain.

- 1 17. The recombinant RSV of claim 7, wherein the phenotypic change
2 comprises a change in viral RNA replication compared to viral RNA replication of a
3 corresponding wild-type or mutant parental RSV strain.
- 1 18. The recombinant RSV of claim 17, wherein accumulation of genomic and
2 antigenomic RNA is decreased compared to accumulation of genomic and antigenomic
3 RNA of the corresponding wild-type or mutant parental RSV strain.
- 1 19. The recombinant RSV of claim 7, wherein the phenotypic change
2 comprises an increase in cumulative mRNA synthesis and a reduction in viral RNA
3 replication compared to cumulative mRNA synthesis and viral RNA replication of a
4 corresponding wild-type or mutant parental RSV strain.
- 1 20. The recombinant RSV of claim 19, wherein a cumulative molar ratio of
2 mRNA to genomic RNA is increased approximately 7- to 18-fold or greater compared to
3 a cumulative molar ratio of mRNA to genomic RNA observed for the corresponding
4 wild-type or mutant parental RSV strain.
- 1 21. The recombinant RSV of claim 7, wherein the phenotypic change
2 comprises a larger plaque phenotype compared to plaque phenotype of a corresponding
3 wild-type or mutant parental RSV strain.
- 1 22. The recombinant RSV of claim 7, wherein the phenotypic change
2 comprises a change in cytopathogenicity compared to cytopathogenicity of a
3 corresponding wild-type or mutant parental RSV strain.
- 1 23. The recombinant RSV of claim 1, wherein the genome or antigenome is
2 further modified by introduction of one or more attenuating mutations identified in a
3 biologically derived mutant human RSV.
- 1 24. The recombinant RSV of claim 23, wherein the genome or antigenome
2 incorporates at least one and up to a full complement of attenuating mutations present
3 within a panel of biologically derived mutant human RSV strains, said panel comprising
4 cpts RSV 248 (ATCC VR 2450), cpts RSV 248/404 (ATCC VR 2454), cpts RSV
5 248/955 (ATCC VR 2453), cpts RSV 530 (ATCC VR 2452), cpts RSV 530/1009 (ATCC

6 VR 2451), cpts RSV 530/1030 (ATCC VR 2455), RSV B-1 cp52/2B5 (ATCC VR 2542),
7 and RSV B-1 cp-23 (ATCC VR 2579).

1 25. The recombinant RSV of claim 23, wherein the genome or antigenome
2 incorporates at least one and up to a full complement of attenuating mutations specifying
3 an amino acid substitution at Val267 in the RSV N gene, Glu218 and/or Thr523 in the
4 RSV F gene, Asn43, Cys319 Phe 521, Gln831, Met1169, Tyr1321 and/or His 1690 in the
5 RSV polymerase gene L, and a nucleotide substitution in the gene-start sequence of gene
6 M2.

1 26. The recombinant RSV of claim 23, wherein the genome or antigenome
2 incorporates at least two attenuating mutations.

1 27. The recombinant RSV of claim 23, wherein the genome or antigenome
2 includes at least one attenuating mutation stabilized by multiple nucleotide changes in a
3 codon specifying the mutation.

1 28. The recombinant RSV of claim 1, wherein the genome or antigenome
2 comprises an additional nucleotide modification specifying a phenotypic change selected
3 from a change in growth characteristics, attenuation, temperature-sensitivity, cold-
4 adaptation, plaque size, host-range restriction, antigen expression, or a change in
5 immunogenicity.

1 29. The recombinant RSV of claim 28, wherein the additional nucleotide
2 modification alters a SH, NS1, NS2, or G gene of the recombinant RSV.

1 30. The recombinant RSV of claim 29, wherein a SH, NS1, NS2, or G gene is
2 deleted in whole or in part or expression of the gene is reduced or ablated by a frame shift
3 or introduction of one or more stop codons in an open reading frame of the gene or a
4 modification of a translational start site.

1 31. The recombinant RSV of claim 28, wherein the nucleotide modification
2 comprises a nucleotide deletion, insertion, substitution, addition or rearrangement of a
3 cis-acting regulatory sequence of a selected gene within the recombinant RSV genome or
4 antigenome.

- 1 32. The recombinant RSV of claim 31, wherein a gene end (GE) signal of the
2 NS1 or NS2 gene is modified.
- 1 33. The recombinant RSV of claim 28, wherein the nucleotide modification
2 comprises an insertion, deletion, substitution, or rearrangement of a translational start site
3 within the recombinant RSV genome or antigenome.
- 1 34. The recombinant RSV of claim 33, wherein the translational start site for a
2 secreted form of the RSV G glycoprotein is ablated.
- 1 35. The recombinant RSV of claim 28, wherein the genome or antigenome is
2 modified to encode a non-RSV molecule selected from a cytokine, a T-helper epitope, a
3 restriction site marker, or a protein of a microbial pathogen capable of eliciting a
4 protective immune response in a mammalian host.
- 1 36. The recombinant RSV of claim 28, wherein the genome or antigenome
2 incorporates a gene or genome segment from parainfluenza virus (PIV).
- 1 37. The recombinant RSV of claim 36, wherein the gene or genome segment
2 encodes a PIV HN or F glycoprotein or immunogenic domain or epitope thereof.
- 1 38. The recombinant RSV of claim 37, wherein the genome segment encodes
2 an ectodomain or immunogenic epitope of HN or F of PIV1, PIV2, or PIV3.
- 1 39. The recombinant RSV of claim 1, wherein the genome or antigenome
2 comprises a partial or complete RSV background genome or antigenome of a human or
3 bovine RSV combined with a heterologous gene or genome segment of a different RSV
4 to form a human-bovine chimeric RSV genome or antigenome.
- 1 40. The recombinant RSV of claim 39, wherein the heterologous gene or
2 genome segment encodes a RSV F, G or SH glycoprotein or an immunogenic domain or
3 epitope thereof.
- 1 41. The recombinant RSV of claim 39, wherein the heterologous gene or
2 genome segment is substituted for a counterpart gene or genome segment in a partial RSV
3 background genome or antigenome

- 1 42. The recombinant RSV of claim 39, wherein the heterologous gene or
2 genome segment is added adjacent to or within a noncoding region of the partial or
3 complete RSV background genome or antigenome
- 1 43. The recombinant RSV of claim 39, wherein the chimeric genome or
2 antigenome comprises a partial or complete human RSV background genome or
3 antigenome combined with a heterologous gene or genome segment from a bovine RSV
- 1 44. The recombinant RSV of claim 39, wherein the chimeric genome or
2 antigenome comprises a partial or complete bovine RSV background genome or
3 antigenome combined with a heterologous gene or genome segment from a human RSV
- 1 45. The recombinant RSV of claim 44, wherein one or more human RSV
2 glycoprotein genes F, G and SH or a genome segment encoding a cytoplasmic domain,
3 transmembrane domain, ectodomain or immunogenic epitope thereof is substituted for a
4 counterpart gene or genome segment within the bovine RSV background genome or
5 antigenome
- 1 46. The recombinant RSV of claim 45, wherein one or both human RSV
2 glycoprotein genes F and G is substituted to replace one or both counterpart F and G
3 glycoprotein genes in the bovine RSV background genome or antigenome.
- 1 47. The recombinant RSV of claim 46, wherein both human RSV glycoprotein
2 genes F and G are substituted to replace counterpart F and G glycoprotein genes in the
3 bovine RSV background genome or antigenome.
- 1 48. The recombinant RSV of claim 45, wherein the heterologous gene or
2 genome segment is from a subgroup A or subgroup B human RSV.
- 1 49. The recombinant RSV of claim 45, wherein the human-bovine chimeric
2 genome or antigenome incorporates antigenic determinants from both subgroup A and
3 subgroup B human RSV.
- 1 50. The recombinant RSV of claim 1 which is a virus.
- 1 51. The recombinant RSV of claim 1 which is a subviral particle.

- 1 52. A method for stimulating the immune system of an individual to induce
2 protection against RSV which comprises administering to the individual an
3 immunologically sufficient amount of the recombinant RSV of claim 1 combined with a
4 physiologically acceptable carrier.
- 1 53. The method of claim 52, wherein the recombinant RSV is administered in
2 a dose of 10^3 to 10^7 PFU.
- 1 54. The method of claim 52, wherein the recombinant RSV is administered to
2 the upper respiratory tract.
- 1 55. The method of claim 52, wherein the recombinant RSV is administered by
2 spray, droplet or aerosol.
- 1 56. The method of claim 52, wherein the recombinant RSV is administered to
2 an individual seronegative for antibodies to RSV or possessing transplacentally acquired
3 maternal antibodies to RSV.
- 1 57. The method of claim 52, wherein the recombinant RSV is attenuated and
2 exhibits increased antigen expression compared to growth and antigen expression of a
3 corresponding wild-type or mutant parental RSV strain.
- 1 58. The method of claim 47, wherein the recombinant RSV elicits an immune
2 response against human RSV A, human RSV B, or both.
- 1 59. An immunogenic composition to elicit an immune response against RSV
2 comprising an immunologically sufficient amount of the recombinant RSV of claim 1 in a
3 physiologically acceptable carrier.
- 1 60. The immunogenic composition of claim 62, formulated in a dose of 10^3 to
2 10^7 PFU.
- 1 61. The immunogenic composition of claim 59, formulated for administration
2 to the upper respiratory tract by spray, droplet or aerosol.

1 62. The immunogenic composition of claim 59, wherein the recombinant RSV
2 exhibits attenuated growth and increased antigen expression compared to growth and
3 antigen expression of a corresponding wild-type or mutant parental RSV strain.

1 63. The immunogenic composition of claim 62 which elicits an immune
2 response against human RSV A, human RSV B, or both.

1 64. An isolated polynucleotide molecule comprising a RSV genome or
2 antigenome which is modified by a partial or complete deletion of M2 ORF2 or one or
3 more nucleotide changes that reduce or ablate expression of M2 ORF2.

1 65. The isolated polynucleotide molecule of claim 64, wherein one or more
2 stop codons are introduced in M2 ORF2.

1 66. The isolated polynucleotide molecule of claim 64, wherein a frame shift
2 mutation is introduced in M2 ORF2.

1 67. The isolated polynucleotide molecule of claim 64 which incorporates NdeI
2 or K5 mutations.

1 68. The isolated polynucleotide molecule of claim 64, wherein the genome or
2 antigenome is further modified by introduction of one or more attenuating mutations
3 identified in a biologically derived mutant human RSV wherein both human RSV
4 glycoprotein genes F and G are substituted to replace counterpart F and G glycoprotein
5 genes in the bovine RSV genome or antigenome.

1 69. The isolated polynucleotide molecule of claim 64, wherein the genome or
2 antigenome comprises an additional nucleotide modification specifying a phenotypic
3 change selected from a change in growth characteristics, attenuation, temperature-
4 sensitivity, cold-adaptation, plaque size, host-range restriction, or a change in
5 immunogenicity.

1 70. The isolated polynucleotide molecule of claim 69, wherein the genome or
2 antigenome is modified by deletion of a SH, NS1, NS2, G gene in whole or in part or by
3 introduction of a frame shift or stop codon in an open reading frame of the gene that
4 reduces or ablates gene expression.

- 1 71. The isolated polynucleotide molecule of claim 70, wherein a SH, NS1,
2 NS2, or G gene is deleted in whole or in part.
- 1 72. The isolated polynucleotide molecule of claim 69, wherein the nucleotide
2 modification comprises a nucleotide deletion, insertion, addition or rearrangement of a
3 cis-acting regulatory sequence of a selected RSV gene within the RSV genome or
4 antigenome.
- 1 73. A method for producing an infectious attenuated RSV particle from one or
2 more isolated polynucleotide molecules encoding said RSV, comprising:
- 3 expressing in a cell or cell-free lysate an expression vector comprising an isolated
4 polynucleotide comprising a recombinant RSV genome or antigenome which is modified
5 by a partial or complete deletion of M2 ORF2 or one or more nucleotide changes that
6 reduce or ablate expression of M2 ORF2, and RSV N, P, L and RNA polymerase
7 elongation factor proteins.
- 1 74. The method of claim 73, wherein the recombinant RSV genome or
2 antigenome and the N, P, L and RNA polymerase elongation factor proteins are expressed
3 by two or more different expression vectors.
- 1 75. An isolated infectious recombinant respiratory syncytial virus (RSV)
2 comprising a RSV genome or antigenome, a major nucleocapsid (N) protein, a
3 nucleocapsid phosphoprotein (P), a large polymerase protein (L), and a RNA polymerase
4 elongation factor, wherein the M2-2 ORF is transposed in the genome or antigenome to a
5 more promoter-proximal or promoter-distal position compared to a native M2-2 gene
6 order position to up-regulate or down-regulate, respectively, expression of the M2-2 ORF,
7 or wherein the M2-2 ORF is incorporated in the genome or antigenome as a separate gene
8 having a gene start and gene end gene end signal to alter expression of the M2-2 ORF.
- 1 76. A method for producing one or more purified RSV protein(s) comprising
2 the steps of:
- 3 infected a host cell permissive of RSV infection with a recombinant RSV having
4 a modification introduced into the genome or antigenome that comprises a M2-ORF 2
5 deletion or knock out mutation under conditions suitable for RSV propagation;

6 isolating the recombinant RSV from the host cell; and
7 purifying said one or more RSV protein(s) to yield a purified RSV protein sample.

1 77. The method for producing one or more purified RSV protein(s) according
2 to claim 76, wherein the purified protein(s) comprises one or more viral antigen(s).

1 78. The method for producing one or more purified RSV protein(s) according
2 to claim 77, wherein the purified protein(s) comprises one or more RSV F and/or G
3 glycoprotein(s) or immunogenic domain(s) thereof.

1 79. The method for producing one or more purified RSV protein(s) according
2 to claim 76, wherein the recombinant RSV expresses one or more viral protein(s) at a
3 level that is approximately 2- to 3-fold greater than a level of expression of said one or
4 more protein(s) by a wild-type or parental mutant RSV.

1 80. The method for producing one or more purified RSV protein(s) according
2 to claim 76, wherein said one or more proteins is/are purified by chromatography using
3 one or more immobilized antibody(ies) that specifically bind(s) to said one or more
4 protein(s).

1 81. The method for producing one or more purified RSV protein(s) according
2 to claim 76, wherein said recombinant RSV is further modified by a mutation that
3 specifies a change to said one or more protein(s) that alters protein immunogenicity,
4 solubility, and/or reactogenicity.

1 82. The method for producing one or more purified RSV protein(s) according
2 to claim 76, wherein said purified RSV protein sample includes a purified RSV G protein.

1 83. The method for producing one or more purified RSV protein(s) according
2 to claim 82, wherein the recombinant RSV is further modified by a mutation that
3 comprises a deletion of an immunopathogenic domain located between amino acids 187
4 and 200 of said RSV G protein.

1 84. The method for producing one or more purified RSV protein(s) according
2 to claim 76, wherein said recombinant RSV is further modified by a mutation that further
3 increases expression of said one or more RSV proteins.

1 85. The method for producing one or more purified RSV protein(s) according
2 to claim 84, wherein said mutation that further increases expression of said one or more
3 RSV proteins includes one or more attenuating mutation(s) identified in a RSV 248/404
4 mutant.

1 86. The method for producing one or more purified RSV protein(s) according
2 to claim 76, wherein said host cell is selected from HEp-2, FRhL-DBS2, MRC, or Vero
3 cells.

1 87. An isolated infectious recombinant respiratory syncytial virus (RSV)
2 comprising a RSV genome or antigenome, a major nucleocapsid (N) protein, a
3 nucleocapsid phosphoprotein (P), a large polymerase protein (L), and a RNA polymerase
4 elongation factor, wherein the genome or antigenome incorporates an amino acid
5 substitution at Asn43 of the RSV polymerase gene L.

1 88. The isolated infectious recombinant RSV of claim 87, wherein Ile is
2 substituted for Asn43.